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STUDIES ON THE CONTROL OF MYELINOGENESIS. 3. SIGNALLING OF OLIGODENDROCYTE MYELINATION BY REGENERATING PERIPHERAL AXONS

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SUMMARY

Continuing from earlier work which demonstrated the peripheral axonal regulation of Schwann cell myelination, this study has investigated the possibility that a peripheral axon can stimulate oligodendrocyte myelination. To test this hypothesis, regenerating PNS axons were allowed to interact with uncommitted oligodendrocytes by transecting a rat peroneal nerve and inserting a segment of the autologous optic nerve between the cut ends. Grafts were maintained for 4–28 weeks and then examined by light and electron microscopy. A few regenerating peripheral myelinated nerve fibers penetrated the optic nerve graft. Some axons penetrated the outer margin of the graft, were myelinated by Schwann cells, and surrounded by astrocyte processes bordered by basal lamina. More centrally in the optic nerve graft, regenerating peripheral axons displayed myelin of CNS type. The outer myelin lamella abutted directly on the plasmalemma surface of surrounding astrocytic processes and was expanded focally to form a glial tongue. These observations demonstrate the experimental induction of central myelination by regenerating peripheral axons and suggest the existence of a common neuronal mechanism to stimulate myelin formation by both the Schwann cell and the oligodendrocyte.

INTRODUCTION

It is well known that two distinct cell types have evolved for the production of myelin in the vertebrate, the oligodendrocyte in the central nervous system (CNS) and the Schwann cell in the peripheral nervous system (PNS). The oligodendrocyte has many cell processes which simultaneously elaborate myelin around multiple CNS axons, while each Schwann cell produces a single internode of peripheral myelin[19]. At

the interface between the peripheral and central nervous systems — the root entry zones of the spinal cord and brain stem — there is an abrupt transition between myelin of PNS and CNS types. This transitional region is characterized by a dome of CNS astrocytes which separates the two populations of myelinating cells and thereby divides the axon into central and peripheral portions[8]. The present study shows that peripheral axons are able to contact and induce oligodendrocytes to elaborate CNS myelin and extends earlier work[3,5,6] which implicated the astrocyte in preventing Schwann cells from migrating into the CNS.

MATERIALS AND METHODS

The experiment was designed to allow PNS axons and Schwann cells to interact with CNS oligodendrocytes and astrocytes. This was accomplished by grafting a segment of an optic nerve between the cut ends of a transected peripheral nerve. Previous peripheral nerve grafting studies[1,21] suggested that regenerating axons would emerge from the proximal stump of the PNS nerve and attempt to penetrate the intervening graft before gaining access to the distal stump of the peripheral nerve. Optic nerve grafts were obtained by unilateral or bilateral eye enucleation in a total of 45 deeply anesthetized Sprague–Dawley rats. Grafts 1.5 mm in length were removed from the shaft of the optic nerve and transplanted at the level of the knee between the cut ends of the peroneal nerve of one limb, or alongside the intact peroneal nerve in the opposite limb as control. The grafts were held in place with an overlying fold of fascia secured with suture thread. Normal optic nerves were also examined in an additional 5 animals. The peroneal-optic grafts and control optic nerve transplants were prepared for light and electron microscopy 4–28 weeks post-operation.

RESULTS

Control optic nerve transplants were viable at all timepoints. By 4 weeks the characteristic fascicular arrangement of the optic nerve had been lost because of astrocytic proliferation. The basal lamina which demarcated the outer astroglial margin in normal nerves was preserved. Degenerating myelin was rare and axons were absent at this time[9,12,23]. Oligodendrocytes remained enmeshed between a complex network of filamentous astrocytic processes. At 8 weeks, but not at 20 weeks, myelin of CNS type (vide infra) was sometimes found around both oligodendrocyte cell bodies and circular astrocytic processes in proximity to these myelinated oligodendrocytes[2]. (This phenomenon has no counterpart in Schwann cells which abruptly cease myelin elaboration following loss of axonal contact[20].)

Peroneal-optic nerve grafts displayed a stereotyped pattern at all time points. The portion originating from the proximal stump of the peroneal contained large diameter (preserved) and small, clustered (regenerating) PNS myelinated fibers. A population of regenerating myelinated and unmyelinated fibers was found in the distal stump portion of the peroneal nerve. Cross-sections of the intervening optic nerve transplant revealed many miniature fascicles containing regenerating PNS fibers disposed circumferentially *outside* of the original graft (Fig. 1). Miniature fascicles

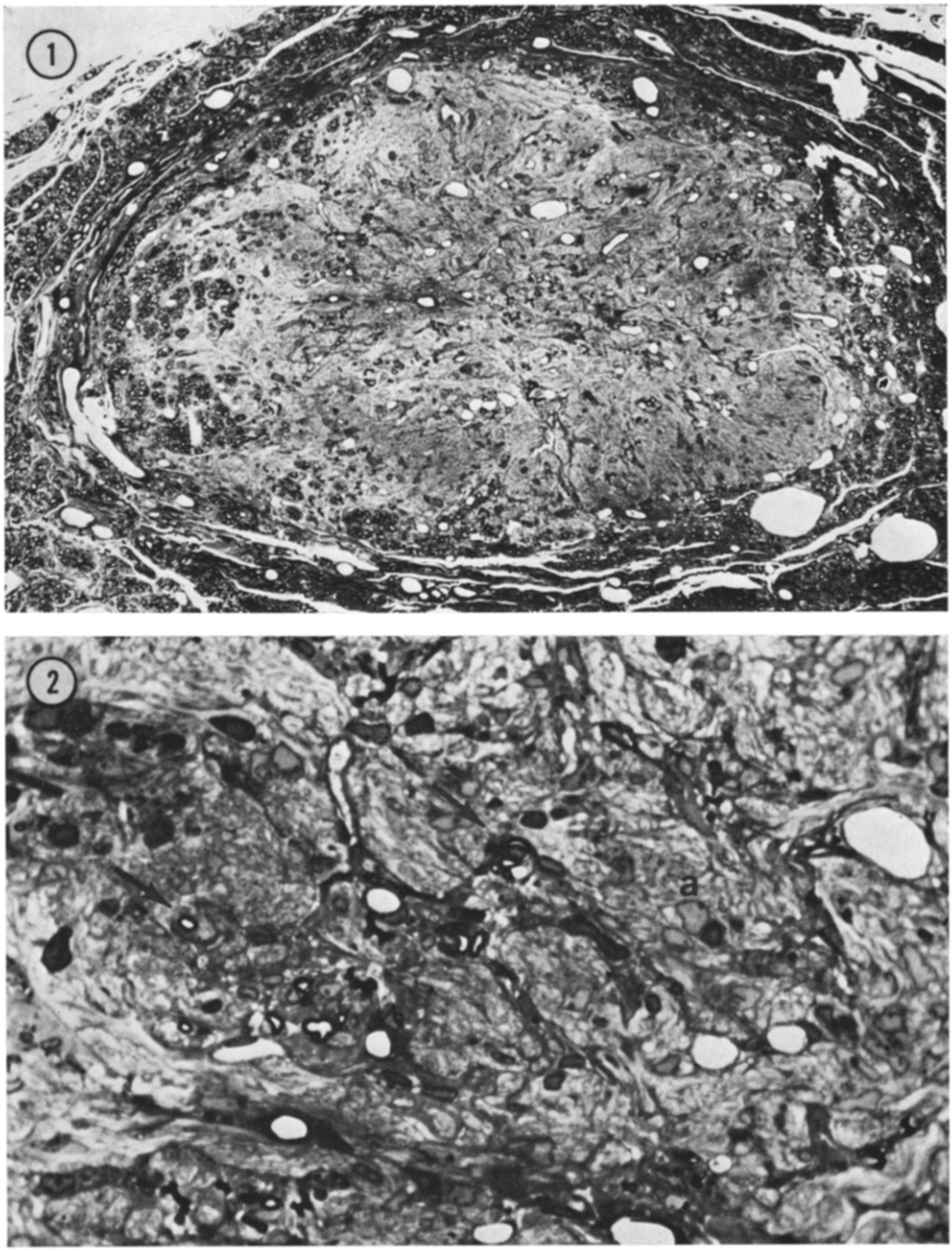

Fig. 1. Cross-section through an optic nerve graft 20 weeks after transplantation. Large numbers of regenerating PNS fibers surround the centrally located graft. × 43.
Fig. 2. Scattered myelinated fibers (arrow) situated deep within the optic nerve graft which is predominantly composed of proliferated astrocytes (a). Clear spaces are patent blood vessels. × 160. Figs. 1 and 2 are light micrographs taken from the same 1 μm epoxy section stained with toluidine blue.

located close to the perimeter of the optic nerve graft sometimes contained an astrocytic bundle enveloping several small unmyelinated axons.

A major observation at 20 weeks was the presence of a few myelinated fibers, each surrounded by proliferated astrocytes *within* the boundary of the original graft (Fig. 2). Two distinct types of myelinated fibers were identified at this site. The outermost fibers were indistinguishable from the PNS fibers located circumferentially outside the original graft; both displayed myelin lamellae with double intraperiod lines and a mean periodicity of 16.1 nm (Figs. 3 and 4). The outer margin of their myelin sheaths was associated with cytoplasm typical of a Schwann cell, a plasmalemma, and an external basal lamina. These PNS fibers were further separated from encircling astrocyte processes by a narrow cleft and the astrocyte basal lamina. The second type

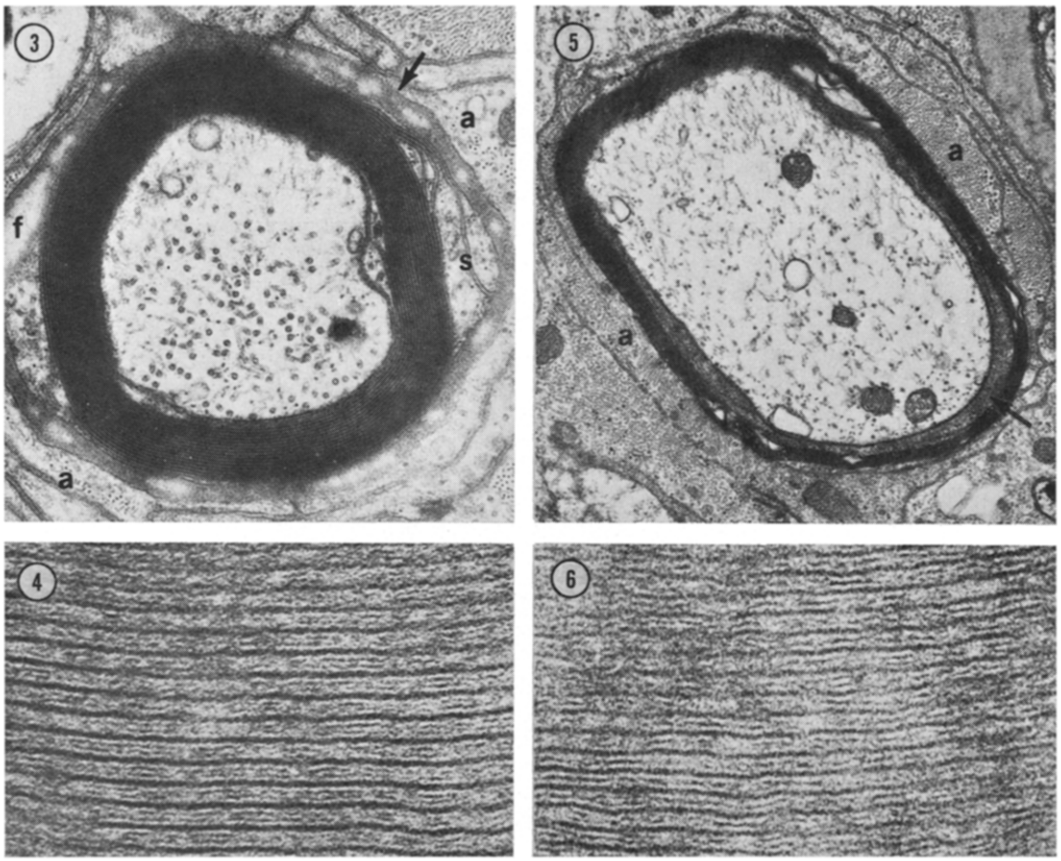

Fig. 3. Regenerating axon with PNS myelin situated within a furrow (f) of a 20-week optic nerve graft. Basal lamina, which borders both the Schwann cell (s) and the astrocytic processes (a), appear fused (arrow) at several positions. × 31,000.

Fig. 4. PNS myelin from fiber comparable to that illustrated in Fig. 3. × 225,000.

Fig. 5. Regenerating axon with CNS myelin situated within the optic nerve graft. The adaxonal cytoplasm of the oligodendrocyte is prominent (arrow) and the outer surface of the myelin sheath abuts directly onto the surfaces of astrocytic processes (a). × 17,000.

Fig. 6. CNS myelin from fiber illustrated in Fig. 5. × 225,000.

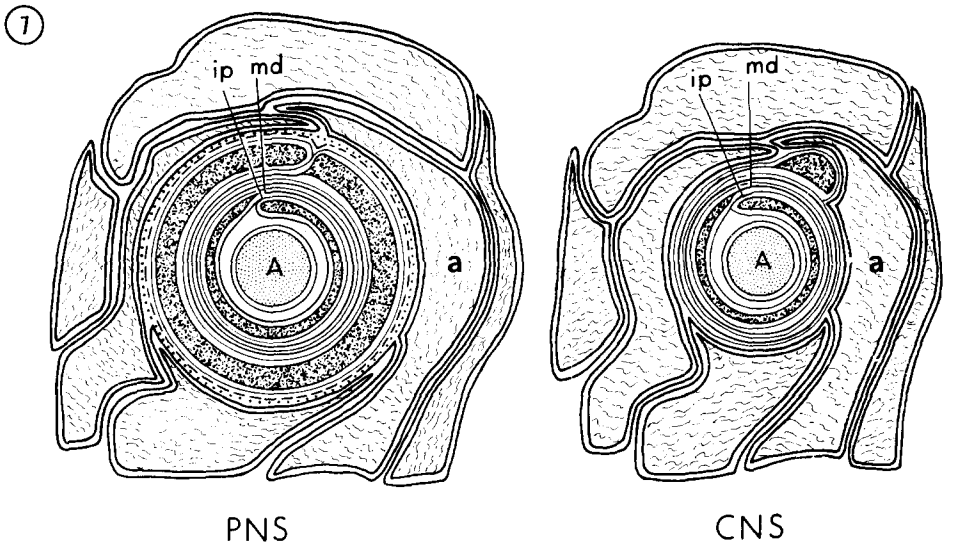

Fig. 7. Diagram defining the relationship of astrocytes (a) to PNS- and CNS-myelinated regenerating axons (A) in the optic nerve graft. The PNS fiber is situated in a narrow channel delimited on one side by the basal lamina of the Schwann cell (broken line) and on the other by a basal lamina bordering the astrocytic processes (dotted line). The major dense lines (md) and intraperiod lines (ip) are also indicated on each fiber.

of myelinated fiber found more centrally within the optic nerve graft displayed myelin of CNS type, with single or, rarely, double intraperiod lines and a mean periodicity of 12.7 nm (Figs. 5 and 6). These fibers measured between 2.3 μm and 3.8 μm and the largest displayed 30 lamellae of CNS myelin. The outermost myelin lamella was focally expanded into a cytoplasmic tongue typical of an oligodendrocyte and abutted onto the plasmalemmae of adjacent astrocytic processes (Fig. 7).

DISCUSSION

These observations demonstrate the experimental induction of oligodendrocyte myelination by regenerating peripheral axons. CNS myelination of PNS axons by naturally ectopic oligodendrocytes has previously been observed in PNS spinal roots and trigeminal nerves of mutant (dystrophic) mice[7,22]. The converse situation — Schwann cells forming PNS myelin around central axons — has also been experimentally induced[4,13]. In addition, myelinating Schwann cells around CNS axons are occasionally found within white matter plaques in multiple sclerosis and in the spinal cord of animals with chronic experimental allergic encephalomyelitis[10,11,16,17]. Since it is also known that neurons regulate Schwann cells to produce myelin[21], the following conclusions may be drawn: (1) peripheral and central axons can each instruct both Schwann cells and oligodendrocytes to elaborate myelin, and (2) the type of myelin manufactured by oligodendrocytes or Schwann cells is cell-specific, and is not related to the location of the myelinating cell in the nervous system. That axons employ a *common* myelinating signal for both Schwann cell and oligodendrocyte may therefore

be indicated. A conservative system of this type would suit the needs of neurons, such as the anterior horn cell, which requires proximal myelination by oligodendrocytes and distal myelination by Schwann cells, With regard to the nature of the myelinating signal, it has been suggested elsewhere[18] that this involves a surface membrane interaction between the axolemma and the plasmalemma of the myelinating cell, and that the quantity of myelin manufactured is positively correlated with the total surface area of axonal contact.

Finally, some conclusions regarding the interaction of astrocytes with axons and myelinating cells may be drawn from these experiments. Firstly, the presence of astrocytes instead of Schwann cells in the envelopment of regenerating unmyelinated PNS axons suggests that the axon surface recognition properties of these two cells are similar. This might account for the pattern found in the CNS–PNS transitional region of the root entry zone where axons are normally enveloped only by astrocytes as they pass from the PNS into the CNS[8]. Secondly, astrocytes may have prevented the entry of Schwann cells into the optic nerve graft, thereby allowing oligodendrocytes to myelinate the otherwise naked regenerating PNS axons. Blakemore has also suggested that, in the spinal cord, astrocytes of the glial limiting membrane serve to prevent the entry of Schwann cells into CNS tissue[3,5]. However, his conclusion that it is the *basal lamina* delimiting the outer surface of these astrocytes which limits Schwann cell migration cannot be supported on the following grounds: (1) in the present study, Schwann cells accompanying the regenerating PNS axons failed to penetrate the cut surface of the optic nerve graft despite the absence of an astrocyte basal lamina, and (2) previous studies have demonstrated that, in normal and regenerated CNS–PNS transition zones, the basal lamina of the terminal Schwann cell is *continuous* with the basal lamina which covers the astrocytes of the glial limiting membranes[14,15]. These observations suggest that it is not the basal lamina of the astrocyte which impedes the movement of Schwann cells into the CNS, but rather that Schwann cell migration is inhibited by contact between the plasmalemmal surfaces of the two cells. A comparable astrocyte-induced inhibition of oligodendrocyte migration from the CNS to the PNS, a possibility suggested by the observed containment of oligodendrocytes within the astrocyte network of optic nerve grafts, would also serve to restrict oligodendrocytes to the spinal cord and thereby prevent their migration to the PNS. Taken in concert, these considerations suggest that astrocytes in the PNS–CNS transition zone play a key role in establishing and maintaining the central and peripheral divisions of the veretebrate nervous system.

NOTE ADDED IN PROOF

Data similar to those reported here have been obtained by Aguayo, A., Dickson, R., Trecarten, J., Aitiwell, M., Bray, J. and Richardson, P., Ensheathment and myelination of regenerating PNS fibers by transplanted optic nerve glia, *Neurosci. Lett.*, in press.

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